DIFFERENTIAL GROWTH OF FALL ARMYWORM LARVAE (LEPIDOPTERA: NOCTUIDAE) REARED ON THREE PHENOTYPIC REGIONS OF WHORL LEAVES FROM A RESISTANT AND A SUSCEPTIBLE MAIZE HYBRID

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ABSTRACT

Two laboratory bioassays were conducted to determine the effect of feeding selected whorl leaf regions of a resistant and a susceptible maize, *Zea mays* L., hybrid on fall armyworm, *Spodoptera frugiperda* (J. E. Smith) growth. In one bioassay, larvae were fed fresh excised whorl leaf tissue and in the other, they were fed reconstituted diets containing ground lyophilized leaf tissue from three phenotypic leaf regions of both hybrids. Results of the two bioassays were similar. Differences in larval weights were found for those larvae fed tissue from leaf regions within and across hybrids. The largest differences among treatments were found within the resistant hybrid, thus showing that regions of the same whorl leaf differ in suitability of food source for larval growth.

Key Words: *Spodoptera frugiperda, Zea mays* L., plant resistance

RESUMEN

Se realizaron dos bioensayos de laboratorio para determinar el efecto en el crecimiento del gusano cogollero del maíz, *Spodoptera frugiperda* (J. E. Smith), al ser alimentado con differentes partes de las hojas del cogollo de una planta de maíz, *Zea mays* L., resistente y de una susceptible. En un experimento, se alimentaron larvas con tejido fresco de hojas del cogollo y, en otro, se alimentaron con dietas reconstituídas que contenían tejido foliar liofilizado de tres regiones fenotípcas de las hojas de ambos híbridos. Los resultados de los dos bioensayos fueron similares. Se encontraron diferencias en el peso larval entre las larvas alimentadas con tejido fresco de diferentes partes de las hojas, ya sea del maíz híbrido o susceptible, y entre las larvas alimentadas con uno u otro tipo de maíz. Las diferencias más grandes entre los tratamientos se encontraron en las larvas alimentadas con hojas del híbrido resistente, mostrando así que regiones de una misma hoja del cogollo difieren entre sí en su calidad como fuente de alimento para el crecimiento larval.

Maize, *Zea mays* L., germplasm lines with leaf feeding resistance to the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) and other lepidopterans [i.e., southwestern corn borer, *Diatraea grandiosella* Dyar; sugarcane borer, *D. saccharlis* (Fab.); and

European corn borer, *Ostrinia nubilalis* (Hübner)] have been released (Davis et al. 1988, Williams & Davis 1989). The primary sources of this resistance were from the Caribbean exotic germplasm Antigua Gpo. 1 and 2 and Republica Dominicana Gpo. 1.

When resistant plants are infested with fall armyworm neonates, fewer larvae survive, and those that do survive weigh less and develop slower than those on similarly infested susceptible plants (Williams et al. 1983, Ng et al. 1985). The mechanisms of resistance responsible for these adverse effects on larval survival and growth are non-preference and antibiosis (Wiseman et al. 1981, 1983). The physical and/or biochemical factors responsible for this resistance are not well understood. Williams & Davis (1997) reviewed the past research conducted to elucidate the factors responsible for the resistance and concluded that a single factor, such as a strong toxin had not been found and that resistance may be conditioned by several factors, such as leaf toughness, increased fiber, and reduced nutritional quality of the resistant plants.

Wiseman & Isenhour (1988) reported differences in weights between fall armyworm larvae feed on green and yellow whorl stage foliage from resistant and susceptible maize. Larvae fed green foliage were larger than those fed the yellow tissue (region of whorl leaf where the larvae normally feed), irrespective of whether the foliage was from a resistant or a susceptible genotype. A preliminary test conducted in our laboratory using different phenotypic regions of whorl leaves from susceptible and resistant maize hybrids as food sources for fall armyworm larvae showed similar growth patterns (F. M. D. unpublished data). We report here on a continuation and expansion of these studies to determine the larval growth responses of fall armyworm when fed on three phenotypic regions of whorl leaves from a selected resistant and susceptible hybrid.

Materials and Methods

Two laboratory bioassays, one using fresh excised whorl leaf tissue and the other lyophilized leaf powder, were conducted to study the growth responses of fall armyworm larvae fed phenotypically different regions of whorl leaves from a susceptible and a resistant maize hybrid. The hybrids selected for this study were Mp707 × Mp708 (resistant) and Ab24E × SC229 (susceptible). The inbreds used to make the resistant hybrid were developed from the Caribbean exotic germplasm (Williams & Davis 1984; Williams et al. 1990a). Plants of each hybrid were grown under field conditions in 10- row blocks. Agronomic practices recommended for our area were used to grow the maize.

The fall armyworm larvae used in these experiments were obtained from our laboratory colony. The procedures used to rear this insect on an artificial diet were described by Davis (1989).

The excised leaf tissue bioassay was begun when the plants reached mid-whorl stage. When plants were needed for feeding larvae, they were cut below the whorl. The whorl and stem portions were then placed in plastic bags by hybrid and maintained in coolers containing ice until processing in the laboratory. The inner whorl leaves were unfurled in the laboratory, and the three selected regions were excised from the leaves. The first region, referred to as green (GR) tissue, was excised from the outer, photo-exposed leaf portion about half way from the tip of the leaf to where the leaf first showed full chlorophyll content. This is a portion of the leaf that larvae normally do not feed on. The second region was within the whorl where the larvae normally feed. This region, referred to as yellow-green (YG) tissue, is just below the upper limits of the surface moisture level within the spirally rolled leaves. The third region was below the YG region. It is referred to as the yellow-white (YW) tissue because it lacks any green color. Larvae do not normally feed this deep within the whorl.

Each excised region was about 5.18 cm wide with about 5.18 cm or more between regions. Leaf mid-ribs were removed from the leaf sections prior to feeding.

Larvae were fed tissue in 8 cm dia. by 8 mm deep round, clear, plastic growth chambers (Bio Quip Products, Gardena, CA). The lid of each chamber contained 13 pinholes for exchange of gases. A 0.64 cm layer of 2% agar plus mold inhibitors (980 ml H_2O + 2.0 g agar + 0.5 g sorbic acid + 0.5 g methyl parasept) was placed in the bottom of each chamber. A piece of circular, autoclaved paper toweling was placed on top of the agar gel. After a few minutes the moisture from the agar wet the paper towel and kept it moist throughout the test period. This allowed leaf tissue placed on the towel to retain its freshness for a few days. After neonates were introduced into each chamber, a strip of autoclaved tissue paper was placed between the chamber's lid and the bottom piece. This was done to prevent neonates from escaping through the pinholes in the chamber's lid. The above description of the growth chamber is a modification of the one used by Wiseman et al. 1981 and Ng et al. 1985.

On the initial day of the experiment, two sections of tissue were placed in each chamber, one on top of the other, to provide an opportunity for the larvae to feed between leaves which is a normal condition within the whorl. Three neonates were placed on the leaf tissue within each chamber using a moistened artist brush. Three days later the number of surviving larvae was recorded and up to 2 of these larvae were removed from the chamber replicates of each treatment and weighed collectively by replication on an electronic balance. These larvae were then discarded. The remaining undisturbed larva in each chamber was fed fresh tissue as needed until the seventh day when a final weight was taken. The larvae were held under environmentally controlled conditions of 27.6°C, 50-60% RH, and a photoperiod of 16:8 (L:D).

The treatments for this bioassay were the two maize hybrids and the three whorl leaf regions of each hybrid. Treatments were arranged in a randomized complete block design with eight replications. Each treatment was represented by larvae in five growth chambers per replication.

During the same time period when whorl tissue was being processed for the excised leaf bioassay, tissue from each phenotypic region was placed in plastic freezer bags and frozen at -18°C for use in the leaf powder bioassay. Later, the frozen samples were removed from the freezer and the tissue was lyophilized, ground to a fine powder, and then returned to the freezer.

On 2 December 1997, a leaf powder diet of each leaf region for the two hybrids was prepared using the following procedure. Agar (3.5 g) was placed in a small boiler with 250 ml of water and brought to a boil. The agar/water solution was then placed into a blender. When the temperature of the solution reached 82°C, 10 g of leaf powder, 528 mg of ascorbic acid, 132 mg of sorbic acid and 132 mg of neomycin sulfate were added to the agar solution and blended for three minutes. This diet recipe is a modification of the one described by Williams et al. (1990b).

After blending, the mixture was poured into 30 ml plastic cups (25) to a depth of about 15 mm each and held under a clean-air hood for 1.5 h to cool and dry. Each cup was infested with one fall armyworm neonate. A paper-board insert cap was used to close the cup. The larvae were maintained in the same environment as described for the excised leaf bioassay.

The six leaf powder diet treatments were arranged in a randomized complete block design with five replications. Each replication consisted of five cups per treatment. Larval weights were obtained 10 and 12 days after infestation.

Mean weights for each treatment were used for statistical analysis of both bioassays. The data were subjected to analysis of variance procedure (SAS 1987) and means were separated by using Fisher's Protected Least Significant Difference test (LSD) [Steel & Torrie 1980].

RESULTS AND DISCUSSION

Excised Leaf Tissue Bioassay

Larval survival was high for all excised leaf tissue treatments. Therefore, antibiosis was not adversely affecting survival.

Significant differences in larval growth rates were clearly evident on both weigh days within and across hybrids (Table 1). Within the phenotypic leaf regions of the resistant hybrid, the order of larval size from largest to smallest was those grown on YW, GR, and YG tissue. The larvae reared on YW tissue were 4.3× and 11.4× larger than those grown on YG tissue on days 3 and 7, respectively. Larvae reared on YG versus GR tissue of the resistant hybrid did not differ in weight on day 3. However, significant differences in weight between these two leaf regions did occur on day 7. 0n this day, the larvae reared on GR tissue weighed 2.5× more than those grown on YG tissue.

Similar differences were observed among the larvae reared on the three phenotypic leaf regions of the susceptible hybrid. But, differences in weights were much less than for larvae reared on similar leaf regions of the resistant hybrid. For example, the larvae reared on YW tissue were only about 2× larger than those reared on YG or GR tissue. No significant difference was found between larvae reared on YG and GR tissues of the susceptible hybrid for both weigh days.

Larval weight comparisons across hybrids are also shown in Table 1. No significant differences were found between larvae reared on YW tissue of the resistant and the susceptible hybrid. However, significant differences were observed between larvae reared on YG tissue of the two hybrids on both weigh days. The larvae reared on YG tissue of the susceptible hybrid weighed 2.6× and 6.1× more than those reared on YG tissue of the resistant hybrid on days 3 and 7, respectively. Larvae fed GR tissue were

TABLE 1. WEIGHTS OF FALL ARMYWORM LARVAE REARED ON THREE PHENOTYPIC REGIONS OF WHORL LEAVES FROM A RESISTANT AND SUSCEPTIBLE MAIZE HYBRID ($\bar{x}$ ± SD).

			Larval weight (mg)	
			—day—	
Hybrid	Classification	Leaf[1] Tissue	3	7
Ab24E × SC229	susceptible	YW	4.8 ± 2.5[2]	275.1 ± 49.2
		YG	2.6 ± 0.6	135.3 ± 13.2
		GR	2.6 ± 0.7	155.4 ± 15.0
Mp707 × Mp708	resistant	YW	4.3 ± 0.9	251.1 ± 40.3
		YG	1.0 ± 0.2	22.1 ± 14.1
		GR	2.0 ± 1.0	55.1 ± 16.4
LSD (0.05) Values:			1.1	29.8

[1]Phenotypic regions of the whorl leaf (YW = yellow-white tissue, YG = yellow-green tissue, and GR = green tissue).

[2]ANOVA values: 3 day weights ($F = 14.71$; df = 5, 35; $P < 0.01$); 7 day weights ($F = 95.44$; df = 5, 35; $P < 0.01$).

only different in weight on day 7 when the larvae grown on the susceptible hybrid tissue weighed 2.8× more than those reared on the resistant tissue.

Leaf Powder Diet Bioassay

Significant differences in larval weights occurred among phenotypic leaf region diets within and across hybrids (Table 2). The biggest differences in weights of larvae grown on the phenotypic leaf region diets of the resistant hybrid were between YG treatment and the other two treatments. Larvae grown on GR and YW tissue diets weighed 10.6× and 6.6×, respectively, more than those reared on YG diet on day 10. About the same degree of differences occurred on day 12 among these treatments. Larvae grown on the resistant hybrid YW tissue weighed about 1.5× more than those reared on GR tissue diet of the same hybrid on both weigh days.

Significant differences in larval weights also were observed among treatment diets within the susceptible hybrid. However, these differences were of a much smaller magnitude than those within the resistant hybrid diet treatments. For example, larvae grown on YW and GR tissue diets were only about 1.4× larger than those reared on YG diet. As with the excised leaf tissue bioassay, large differences in larval weights occurred between the resistant and susceptible hybrid for those fed YG tissue diets. At both weigh days larvae grown on the susceptible YG diet weighed 7.9× more than those on YG diet of the resistant hybrid. Significant, but smaller differences were detected between GR leaf tissue diets of the two hybrids. No significant differences in larval weights were detected between those fed YW tissue diets of the susceptible and resistant hybrid.

Results from experiments using excised leaf tissue and leaf powder diet bioassays were similar. Differences in larval weights occurred among leaf tissue regions within

TABLE 2. WEIGHTS OF FALL ARMYWORM LARVAE REARED ON LYOPHILIZED LEAF POWDER DIETS FROM THREE PHENOTYPIC REGIONS OF WHORL LEAVES FROM A RESISTANT AND SUSCEPTIBLE MAIZE HYBRID ($\bar{x}$ ± SD).

			Larval weight (mg)	
			—day—	
Hybrid	Classification	Leaf[1] Tissue	10	12
Ab24E × SC229	susceptible	YW	87.7 ± 12.3[2]	136.1 ± 19.0
		YG	61.9 ± 10.7	130.5 ± 18.4
		GR	110.0 ± 15.7	240.0 ± 32.2
Mp707 × Mp708	resistant	YW	82.3 ± 19.2	167.1 ± 24.8
		YG	7.8 ± 1.4	16.5 ± 3.0
		GR	51.3 ± 7.3	120.8 ± 17.5
LSD (0.05) Values:			14.8	27.5

[1]Phenotypic regions of the whorl leaf (YW = yellow-white tissue, YG = yellow-green tissue, and GR = green tissue).

[2]ANOVA values: 10 day weights ($F = 50.12$; df = 5, 20; $P < 0.01$); 12 day weights ($F = 60.74$; df = 5, 20; $P < 0.01$).

and across the susceptible and the resistant maize hybrid. Our results were generally in agreement with those reported by Wiseman and Isenhour (1988).

The most interesting result was within the resistant hybrid whorl leaf, where suitability of food for larval growth varied from excellent (YW tissue) to very poor (YG tissue) to moderately poor (GR tissue). Thus, the resistant factor(s) must not be operative in YW tissue, but are present in YG and GR tissues. It is also interesting that larvae fed on the resistant hybrid GR tissue weighed significantly more than those fed YG tissue of the same hybrid thus, indicating a shift in intensity of resistance.

Our findings provide us with a better understanding of the susceptible and resistant whorl leaf as it relates to fall armyworm growth, and to the presence and intensity of resistance factors. Also, our results provide a new opportunity for determining the factor(s) responsible for the resistance as biophysical and biochemical characters can be compared now within the phenotypic whorl leaf regions of resistant genotypes as well as across genotype comparisons (resistant versus susceptible). This study also shows the importance of using the appropriate natural larval feeding site within the plant's whorl when conducting laboratory leaf bioassays. Using the wrong leaf region could result in incorrect conclusions.

ENDNOTE

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